



Atty. Dkt. No. 053466-0274

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Masayuki TSUCHIYA  
Title: NATURAL HUMANIZED ANTIBODY  
Appl. No.: 09/509,098  
Filing Date: 03/22/2000  
Examiner: Larry Ronald Helms  
Art Unit: 1642

**LETTER REQUESTING ACKNOWLEDGEMENT**

Mail Stop ISSUE FEE  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

In reviewing the above-captioned application file upon payment of the Issue Fee, the undersigned has noted that Applicant's Amendment under 37 C.F.R. §1.312 submitted on August 8, 2005, has not been considered by the Examiner.

The Applicant respectfully requests entry of this amendment under Rule 312, a copy of this Amendment is also enclosed for the Examiner's convenience.

Respectfully submitted,

Date September 15, 2005

By

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Title: NATURAL HUMANIZED ANTIBODY

Inventor(s): Masayuki TSUCHIYA

Dkt. No. 053466-0274

Appl. No.: 09/509,098

HCW (8/8/05)

- 312 Amendment (5 pgs.).

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Due Date:

Date Filed: August 8, 2005

Attorney Initials: HCW/MEMU:mb

Insp. By:

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Masayuki TSUCHIYA

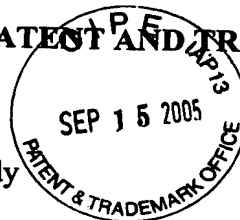
Title: Natural Humanized Antibody

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**AMENDMENT UNDER 37 CFR § 1.312**

MAIL STOP AF  
Commissioner for Patents  
PO Box 1450  
Alexandria, Virginia 22313-1450

**COPY**

Sir:

**Amendments to the Claims** are reflected in the listing of claims beginning on page 2 of this document.

**Remarks/Arguments** begin on page 4 of this document.

**Amendments to the Claims**

This listing of claims will replace all prior versions, and listings, of claims in the application.

1-13. (Cancelled)

14. (Currently amended) A method for preparing a humanized antibody, wherein a framework region ("FR") in the humanized antibody is a FR naturally occurring in human antibodies, comprising the steps of:

- (1) obtaining a humanized antibody, wherein the humanized antibody has:
  - i) six complementary determining regions ("CDRs") of a first animal species; and
  - ii) eight FRs of a second animal species, wherein one or more amino acid residues in one or more of the FRs have been substituted to retain antigen binding ability, with corresponding amino acid residues in FRs of the first animal species, and wherein said second animal species is human;
- (2) conducting a homology search using a database of amino acid sequence of FRs naturally occurring in human antibodies ("natural FRs") in comparison with the amino acid sequence of the humanized antibody obtained in step (1), wherein the homology search is conducted over all 8FRs;
- (3) preparing a list of amino acid sequences of the natural FRs having the same as or at least 80% homology with the amino acid sequence of the FR, of the humanized antibody obtained in step (1),
- (4) selecting, from the list of step (3), a natural FR which has
  - i) at corresponding positions the same amino acid residues as the amino acid residues introduced by the substitution in step (1); and
  - ii) comprises an amino acid sequence that is the same as or has at least 80% homology with the FR sequence of the humanized antibody obtained in step (1);

(5) if the amino acid sequence of the FR, in which amino acid residues have been substituted in step (1), of the humanized antibody obtained in step (1) has one or more amino acid residues that are different from amino acid residues at corresponding positions of the natural FR selected in step (4), replace said different amino acid residues in the FR sequence of the humanized antibody obtained in step (1) with corresponding amino acid residues in the natural FR;

(6) constructing an expression vector expressing an amino acid sequence of the antibody obtained via steps (1) to (5);

(7) culturing cells comprising an expression vector constructed in step (6); and

(8) recovering from the culture the humanized antibody comprising the natural FR and 6 CDRs from the first animal species and wherein the recovered humanized antibody binds the same [[an]] antigen that [[is the same as]] the antibody from the first animal species binds.

15. (Previously presented) The method according to claim 14, wherein the first animal species is a non-human mammal.

16 - 17. (Canceled)

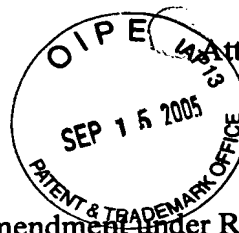
18. (Previously presented) The method according to claim 15, wherein the non-human mammal is selected from a mouse, rat, hamster, rabbit and monkey.

19. (Previously presented) The method according to claim 14, wherein all natural FRs belong to the same subgroup.

20. (Previously presented) The method according to claim 14, wherein the number of the substituted amino acid residues of the FR in step (1) is from one to ten.

21. (Previously presented) The method according to claim 14, wherein the number of the different amino acid residues in the FR in step (5) is from one to ten.
22. (Previously presented) The method according to claim 14, wherein the substituted amino acid residues in the FR in step (1) comprise an amino acid residue selected from amino acid residues responsible for canonical structure of the antibody, amino acid residues involved in the maintenance of the structure of CDRs and the amino acid residues that directly interact with an antigen.
23. (Previously presented) The method according to claim 20, wherein the substituted amino acid residues in the FR in step (1) comprise an amino acid residue selected from an amino acid residue at position 71 of the heavy chain or at position 94 of the heavy chain.

REMARKS



Applicant respectfully requests entry of this amendment under Rule 312, which merely clarifies the claims and does not require prosecution to be reopened. The current claim amendment is the result of an agreement reached in a telephone conversation on August 2, 2005 with Examiner Helms and the undersigned attorney.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

If any fees are due in connection with the filing of this Amendment, please charge the fees to our Deposit account No 19-0741. If a fee is required for an extension of time under CFR § 1.136 that is not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Date August 5, 2005

By

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